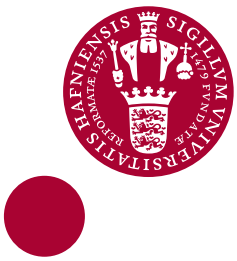

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Heritability of Retinal Vascular Fractals: A Twin Study

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Purpose. To determine the genetic contribution to the pattern of retinal vascular branching expressed by its fractal dimension.

Methods. This was a cross-sectional study of 50 monozygotic and 49 dizygotic, same-sex twin pairs aged 20 to 46 years. In 50°, disc-centered fundus photographs, the retinal vascular fractal dimension was measured using the box-counting method and compared within monozygotic and dizygotic twin pairs using Pearson correlation coefficients. Falconer's formula and quantitative genetic models were used to determine the genetic component of variation.

Results. The mean fractal dimension did not differ statistically significantly between monozygotic and dizygotic twin pairs (1.505 vs. 1.495, $P = 0.06$), supporting that the study population was suitable for quantitative analysis of heritability. The intrapair correlation was markedly higher (0.505, $P = 0.0002$) in monozygotic twins than in dizygotic twins (0.108, $P = 0.46$), corresponding to a heritability h^2 for the fractal dimension of 0.79. In quantitative genetic models, dominant genetic effects explained 54% of the variation and 46% was individually environmentally determined.

Conclusions. In young adult twins, the branching pattern of the retinal vessels demonstrated a higher structural similarity in monozygotic than in dizygotic twin pairs. The retinal vascular fractal dimension was mainly determined by genetic factors, which accounted for 54% of the variation. The genetically predetermination of the retinal vasculature may affect the retinal response to potential vascular disease in later life.

Keywords: retinal vascular fractal dimension, heredity, twin, monozygotic, dizogotic

The pattern of retinal blood vessels in human varies considerably between individuals.[1] The significance of these variations for health and disease is largely unknown, as is the extent to which they are governed by genetic factors or environmental and lifestyle influences. The retinal blood vessels are the only part of the human vasculature available for direct in vivo inspection. It is possible to perform direct, noninvasive examinations of the geometrical features of the retinal vascular tree using recently developed computer-based software.[2]

Fractal patterns are well known in nature. They are found in branching structures like frost crystals, lightening, and tree branches. They are characterized by a self-similar pattern that is unaffected by the magnitude of the view. In other words, as studied under a different magnification a smaller part of the whole will have the same structure as the larger part. By specialized software it is possible to measure the fractal pattern of the retinal vascular tree.[3] Retinal vascular fractals are measured by the fractal dimension, defined as a noninteger unit between 1 and 2 that increases correspondingly to the density of the retinal vascular tree. The retinal fractal dimension has a high intereye agreement in healthy subjects,[4] and even though it generally decreases with aging[4] it is fairly resistant to metabolic changes as measured by different levels of diabetic retinopathy.[5]

Studies have demonstrated that the retinal vascular fractals are linked to ocular and systemic diseases like diabetic retinopathy,[6–8] stroke,[9,10] and Alzheimer's disease.[11] For instance, the retinal vascular fractal dimension not only correlates to, but also predicts long-term proliferative diabetic retinopathy and other microvascular complications in type 1 diabetes.[12,13] It is, however, not evident what defines the structure of the retinal vascular tree. Hence, the aim of the present study was to determine the relative influence of genetic factors on the retinal vascular fractal dimension in a cohort of young, Danish twins.

Methods

Subjects

We examined 59 monozygotic and 55 dizygotic same-sex twin pairs aged 20 to 46 years from the study 'The importance of genes, familiar and common environment for the development of insulin resistance, abdominal adiposity and cardiovascular risk factors (GEMINAKAR)'. All subjects for this study were

recruited from the population-based Danish Twin Registry[14] that includes more than 85,000 twin pairs in birth cohorts from 1870 to 2009. The ascertainment rate of the registry was 90% up to March 1968 and 100% after April 1968, when a fully comprehensive computerized national population database was introduced (Danish Civil Registry). In the present study, zygosity was determined using nine polymorphic DNA-based microsatellite markers (AmpFISTR Profiler Plus Kit; Perkin Elmer Applied Biosystems, Foster City, CA, USA). This principle has an error probability of 0.003% or lower.[15]

The twins were invited by a mailed questionnaire.[16] Exclusion criteria were: pregnancy, breastfeeding, known diabetes or cardiovascular disease, and known conditions preventing the completion of an ergometric bicycle test. Of 2099 potential pairs, 764 pairs were eligible and willing to participate. Randomized exclusions were made in specific age groups to achieve a uniform age distribution, reducing the participant number to 621. Of these, twin pairs where both twins lived on the island of Zealand were invited to participate in a separate ophthalmic examination, for which 114 pairs volunteered. Previously published data on the cohort include lens autofluorescence,[17] retinal nerve fiber layer thickness,[18] optic disc diameter,[19] retinal vessel diameter,[20] tortuosity of retinal vessels,[1] the presence of small hard drusen,[21] and the presence of cilioretinal arteries.[22]

All participants gave their written informed consent. The study was approved by the regional medical ethics committee and followed the tenets of the Helsinki Declaration.

Procedures

Subjects responded to a detailed questionnaire including information about lifelong smoking habits. Study examinations that have previously been described in detail included oral glucose tolerance testing, blood pressure measurement, blood sampling, and measurement of height and weight.[16–18,22] The ophthalmic examination included subjective refraction, determination of Snellen visual acuity, pupil dilation (with phenylephrine hydrochloride 10% and tropicamide 1%), slit-lamp biomicroscopy, and nonstereoscopic digital greyscale fundus photography. A Topcon TRC-50X (Topcon, Tokyo, Japan) equipped with a digital back piece (MEGAPLUS model 1.4; Eastman Kodak, San Diego, CA, USA) was used to capture four 50° fields and one 20° optic disc-centered photograph in red-free illumination (1024 × 1024 pixels, filter: Wratten 54; Eastman Kodak). These images were used to rule out glaucoma or retinal disease.

Retinal vascular fractal analysis was performed using the disc-centered, 50° photographs. Grading was performed by a trained grader (ASV) using the semiautomatic computer software Singapore Institute Vessel Assessment-Fractal (SIVA-Fractal), version 1.0 (School of Computing, National University of Singapore, Singapore), by a standardized protocol that has previously been validated with high intra- and intergrader reliability.[23,24] The software automatically detected all vessels coursing through a 0.5- to 2.0-disc diameter zone from the disc margin. The program provided skeletonized line tracing of the vasculature, and artifacts (i.e., choroidal vessels and pigment abnormalities) were then removed by the grader. Finally, the fractal dimension was calculated by the program using the box-counting method, which is a well-established method for structures not perfectly self-similar, such as the retinal vasculature.[25,26] For all participants, the right eye was graded and used for the analyses. The left eye was not used for analysis, given that no twin pairs had at least one ungradable retinal image in the right eye but gradable images in left eyes in both twins.

Table 1. Characteristics of the Study Population of 99 Same-Sex Twin Pairs

	Monozygotic Twin Pairs, $n = 50$	Dizygotic Twin Pairs, $n = 49$	*P* Value
Number of participants	100	98	
Men/women, %	48/52	41/59	0.27*
Age, y	36 ± 12	36 ± 10	0.95†
Systolic blood pressure, mm Hg	115 ± 13	117 ± 11	0.34‡
Diastolic blood pressure, mm Hg	70 ± 10	70 ± 8	0.88‡
Fasting plasma glucose, mM	4.89 ± 0.45	4.89 ± 0.41	0.90‡
Smoking, # of pack y	0 ± 5.5	0 ± 9.0	0.27†
Body mass index, kg/m^2	23.5 ± 3.6	23.7 ± 3.5	0.73‡
Height, cm	1.74 ± 0.09	1.74 ± 0.09	0.83‡
Weight, kg	71.7 ± 12.6	72.2 ± 13.3	0.82‡
Physical fitness, mL O_2, kg, min	37.2 ± 6.57	34.9 ± 7.76	0.07‡
Total cholesterol, mM	5.19 ± 1.05	5.57 ± 1.06	0.10‡
Low-density lipoprotein, mM	3.14 ± 0.96	3.36 ± 1.01	0.28‡
Very low-density lipoprotein, mM	0.4 ± 0.2	0.4 ± 0.2	0.09†
High-density lipoprotein, mM	1.50 ± 0.35	1.55 ± 0.47	0.44‡
Triglycerides, mM	1.0 ± 0.5	1.3 ± 0.8	0.15†
Retinal vascular fractal dimension	1.505 ± 0.029	1.495 ± 0.032	0.06‡

Data are presented as mean ± SD except for age, smoking, very low-density lipoproteins, and triglycerides that are presented as median ± interquartile range due to a skewed distribution. The individual measurements were used to calculate the standard deviations and interquartile ranges whereas the mean of each twin pair was used when calculating *P* values.

* χ^2 test.

† Median two-sample test.

‡ Student's two-tailed, unpaired *t*-test.

Statistics

Standard deviations and interquartile ranges (for skewed distributions) were calculated using data from both members of the twin pairs to describe the variation in the data. We used visual inspection of the distributions for assessing normality. To account for dependency of the data within twin pairs, the average values of the twin pairs were used when comparing the groups of monozygotic and dizygotic twins with two-sampled, two-tailed Student's *t*-test or median two-sample test (skewed distributions) (Table 1). We compared the retinal vascular fractal dimension within monozygotic and dizygotic twin pairs by Pearson's correlations (Fig. 1).

The classical twin data model is based on the assumption that monozygotic twins have identical genotypes, and that all observed differences between twins in a pair are caused by environmental factors. On the other hand, dizygotic twins only share 50% on average of their genes. The extent to which monozygotic twins are more alike than dizygotic twins is therefore assumed to reflect additional genetic sharing. The proportion of total variation attributable to genetic factors is expressed as heritability (h^2), which is twice the difference in interclass correlation (r) between monozygotic and dizygotic twins.[27]

$$h^2 = 2(r_{mz} - r_{dz}) \quad (1)$$

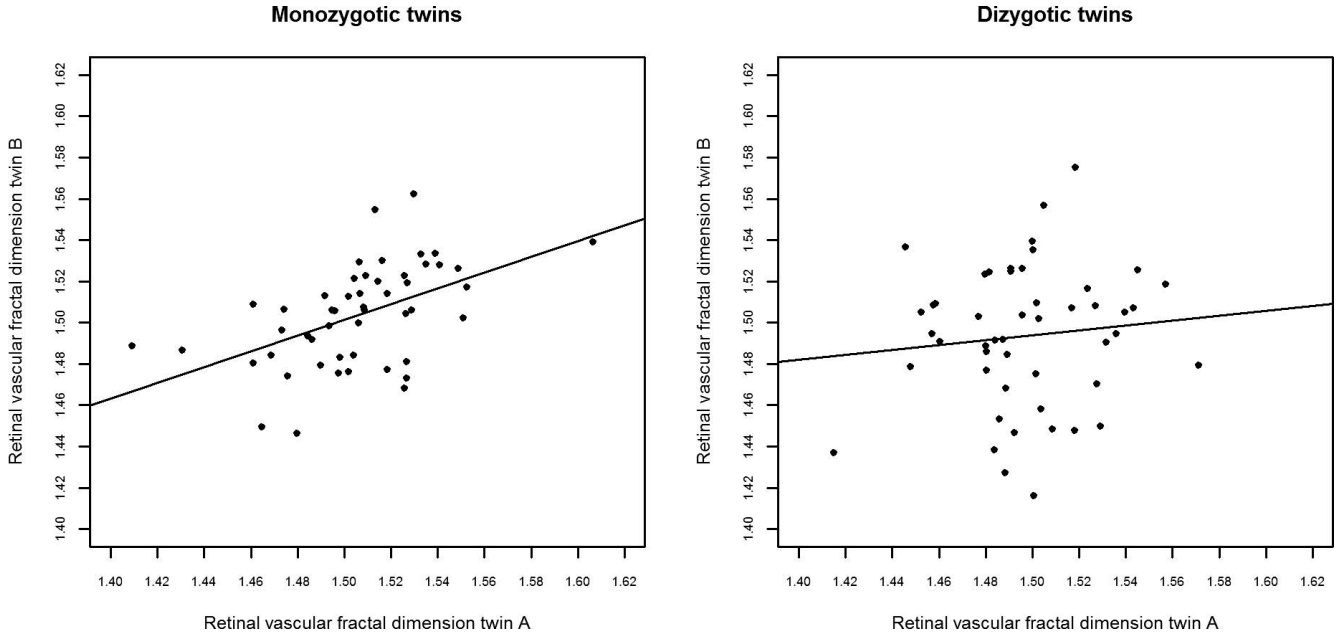

Figure 1. Illustration of the intrapair correlation between twin A and twin B in the retinal vascular fractal dimension in 50 monozygotic twin pairs (*left-hand side*) and 49 dizygotic twin pairs (*right-hand side*). The Pearson's correlation coefficient was 0.505 ($P = 0.0002$) for monozygotic twins and 0.108 ($P = 0.46$) for dizygotic twins resulting in a heritability h^2 of 0.79 calculated using Falconer's formula.

where r is defined as

$$r = \frac{covar_{(twinA,twinB)}}{\sqrt{(var_{(twinA)} \times var_{(twinB)})}} \quad (2)$$

Furthermore, we applied quantitative genetic models splitting the variation of retinal vascular fractal dimension into A (additive genetic effects), D (dominant genetic effects), C (common environmental effects), and E (individual environmental effects). A, D, and C cannot be estimated simultaneously, and hence we applied a series of models and compared their fit by the Akaike Information Criterion (AIC).[28] We applied those models both unadjusted as well as adjusted univariately for systolic blood pressure and cholesterol. It has to be noted that while h^2 implicitly allows a negative effect of common environment, the quantitative genetic models force all four components (A, D, C, and E) to be nonnegative.

Data analysis was made with SAS 9.4 software package (SAS Institute, Cary, NC, USA) as well as R 3.3.1 with the package mets.[29,30]

Results

Of the 59 monozygotic and 55 dizygotic twin pairs available, the present study excluded nine monozygotic twin pair and six dizygotic twin pairs because retinal fundus photographs were not present or not gradable in either eye of at least one twin within the pair. Thus, the study included 50 monozygotic and 49 dizygotic twin pairs.

Table 1 describes the clinical characteristics of the twin pairs of the study according to zygosity. In the entire population, the mean retinal vascular fractal dimension (with SD) was 1.500 (0.0309) with a trend toward a higher value in monozygotic than dizygotic twins (1.505 vs. 1.495, $P = 0.06$).

The interpair correlations for the fractal dimension are presented in Figure 1. The Pearson correlation coefficient was 0.505 ($P = 0.0002$) for monozygotic twins, and 0.108 ($P = 0.46$) for dizygotic twins. Based on this we calculated a heritability h^2 for the fractal dimension of 0.79 (Fig. 1).

The results of the quantitative genetic models are presented in Table 2. The best fitting model, both unadjusted and adjusted, was the model only consisting of dominant genetic (D) and individual environmental (E) components with an estimated 54% of the variation explained by dominant genetic effects. As a model with dominant genetic (D) components without additive genetic (A) components might be regarded as biologically implausible, we also investigated the ADE-model, resulting in an estimated 0% of A, and hence the same results as the AD-model, albeit with a worse AIC. The difference between these estimates of 54% and the h^2 of 0.79 can be explained by the implicit negative effect of common environment present in this estimate of h^2 due to a much stronger association in monozygotic than in dizygotic twins.

Discussion

In the present cross-sectional study of young Danish monozygotic and dizygotic twins, we demonstrated that 54% of the total variation in the retinal vascular fractal dimension was determined by genetic factors.

While the heritability of the retinal fractal dimension has not previously been investigated, the retinal vascular caliber is another morphologic, noninvasive marker of the retinal vessels that has been studied in twins. In persons aged 5 to 90 years, Sun et al.[31] evaluated the heritability of 374 monozygotic and 536 dizygotic twin pairs as well as 322 siblings from the Twins Eye Study in Tasmania and the Brisbane Adolescent Twin Study. The hereditability of the retinal arteriolar caliber was 59.4% and 56.5% for the two cohorts, and corresponding values for the venous caliber were 61.7% and 64.2%. In comparison, a higher heritability was found for arterioles (70%) and venules (83%) in a previous study of the present cohort.[20] Differences between the Australian and Danish results might be due to different measurements of retinal caliber or different age and genetic composition of the study populations.

The finding that the distribution of retinal vessels is predominantly determined by heredity does not mean that the characteristics are necessarily congenital, or that they are

Table 2. Results of the Quantitative Genetic ADCE-Modeling For Heritability (With 95% Confidence Intervals) of Retinal Vascular Fractal Dimension in a Crude Model and a Model Adjusted for Systolic Blood Pressure and Serum Cholesterol

Model	A	D	C	E	AIC
Crude model, $N = 198$					
ACE	0.49 (0.28, 0.71)		0	0.51 (0.29, 0.72)	−822.1250
ADE	0	0.54 (0.34, 0.73)		0.46 (0.27, 0.66)	−823.5531
DCE		0.54 (0.34, 0.73)	0	0.46 (0.27, 0.66)	−823.5531
AE	0.49 (0.28, 0.71)			0.51 (0.29, 0.72)	−824.1250
DE		**0.54 (0.34, 0.73)**		**0.46 (0.27, 0.66)**	**−825.5531**
CE			0.29 (0.11, 0.47)	0.71 (0.53, 0.89)	−819.1078
Model adjusted for systolic blood pressure and s-cholesterol ($N = 189$)					
ACE	0.49 (0.43, 0.55)		0	0.51 (0.45, 57)	−746.3294
ADE	0	0.54 (0.46, 0.62)		0.46 (0.38, 0.54)	−747.8397
DCE		0.54 (0.46, 0.62)	0		−747.8397
AE	0.49 (0.43, 0.55)			0.51 (0.45, 0.57)	−748.3294
DE		**0.54 (0.46, 0.62)**		**0.46 (0.38, 0.54)**	**−749.8397**
CE			0.28 (0.20, 0.35)	0.72 (0.65, 0.80)	−743.6890

Data from a crude and an adjusted quantitative genetic model splitting the variation of the retinal vascular fractal dimension into A (additive genetic effects), D (dominant genetic effects), C (common environmental effects), and E (individual environmental effects). The fits of the models were estimated by the Akaike Information Criterion (AIC). The best fitting models have been indicated by bold.

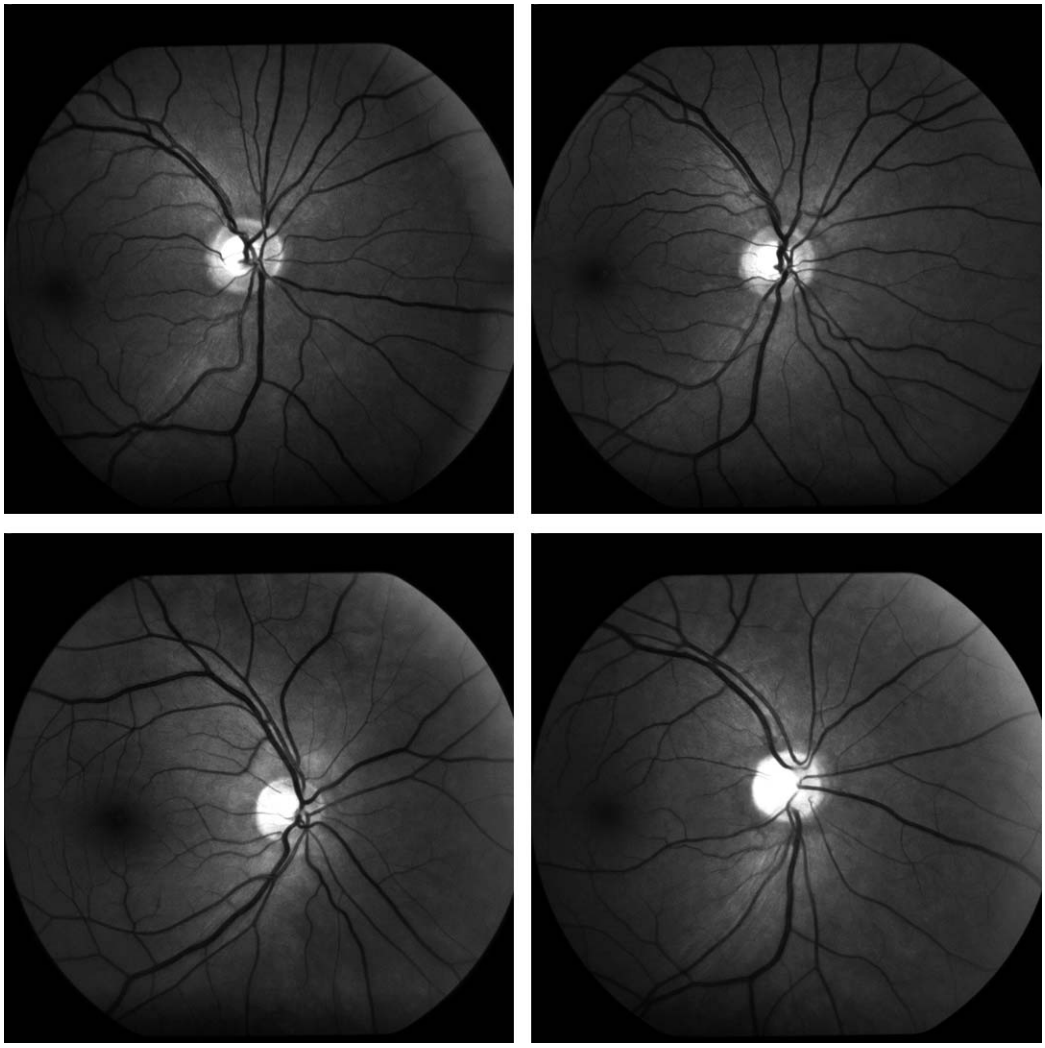

Figure 2. Disc-centered images of retinal vasculature in twin A (*left-hand side*) and twin B (*right-hand side*) in a monozygotic (*upper*) and dizogotic (*lower*) twin pair. The retinal vascular fractal dimensions for twin A and B were 1.508 and 1.507 in the monozygotic and 1.509 and 1.448 in the dizogotic twin pair, respectively, corresponding to an intrapair difference of 0.07% and 4.00%. This illustrates the higher variability in the retinal vascular fractal dimension, which was generally found in dizogotic twin pairs.

intrinsic vascular characteristics. Vascular patterns vary with extraocular factors, such as blood pressure,[32] and hematocrit,[33] and with local factors, such as retinal vein occlusion,[34] uveitis,[35] and diabetic retinopathy.[36] Consequently, the heritability of the retinal vascular fractal dimension could be mediated by other factors than the heritability of vascular characteristics, especially such systemic factors as the heritability of blood pressure control mechanisms. Our estimate of the genetic component of the variability of the fractal dimension did not change after adjusting for systolic blood pressure and serum cholesterol, indicating that the genetic component was not mediated by these variables.

It is assumed that the design of the retinal vascular tree follows the optimization principle,[3] based on the theory of minimum work. This was originally postulated by Murray in 1926,[37] and has later been validated for the retinal tree.[38,39] Given that the retinal vascular fractals are associated with subclinical structural changes in early diabetic retinopathy[8] and also predicts long-term microvascular complications,[13] it seems plausible that subtle changes in the retinal vascular system may be an early indicator of upcoming disease.

Other studies of the same cohort confirm that the retinal structure is mainly derived from heritability. For instance, 77% of the variation of the optic disc dimension could be explained by additive genetic factors,[19] and the heredity of the retinal nerve fiber layer thickness was 82%.[18]

It was a strength of the present study that participants were well-characterized genetically and that retinal vascular fractals were measured using a well-established, highly-reliable method. On the other hand, the relatively small sample size limits the generalizability of the study, and in addition we were not able to address specific genes responsible for the structural variations described.

In this study of well-characterized twin pairs, we demonstrated a genetic effect for the variation in the retinal vascular fractal dimension. Upcoming longitudinal studies may address morphologic changes throughout life and the potential effect of genes and environment in this process.

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